REMARKS/ARGUMENTS

Applicants thank Examiner Nguyen for the telephonic interview held on March 15, 2004. During this interview, a number of issues were clarified and a number of amendments to the claims were proposed that have helped Applicants to more fully address the concerns of the Examiner. The issues discussed during this interview will be set forth in greater detail below.

Claims 1-3 and 5-67 are pending in the above-referenced patent application; claims 1-3, 6-17, 19-30, 32-35, 37-48, 50-61 and 62 are currently under examination. In order to expedite prosecution, independent claims 1 and 33 have been amended in accordance with the Examiner's suggestions to expressly recite (1) that the nucleic acid is encapsulated in the lipid; and (2) that the conjugated lipid that inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid, an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula

A——W——Y

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wherein: A is a lipid moiety; W is a hydrophilic polymer; and Y is a polycationic moiety. Support for the amendments to the claims can be found throughout the specification and claims as originally filed and, thus, no new matter has been introduced.

Claims 1-3, 6-15, 17, 19-22, 27-30, 32-35, 37-46, 48-53, 58-61 and 63 remain rejected under 35 U.S.C. § 103(a) as allegedly being obvious. Claims 1-4, 6-15, 17, 19-22, 27-30, 32-35, 37-46, 48-53, 58-61 and 63 remain rejected under the judicially created doctrine of obviousness-type double patenting. For the reasons discussed with the Examiner during the telephonic interview and set forth herein, each of these remaining rejections is overcome.

I. The Invention

The present invention provides novel and surprisingly effective methods for delivering nucleic acids to cells that employ the use of nucleic acid-lipid particles comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a nucleic acid, wherein the nucleic acid is encapsulated in the lipid, and wherein the conjugated lipid that

inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid, an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula

A——W——Y

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wherein: A is a lipid moiety; W is a hydrophilic polymer; and Y is a polycationic moiety; and an endosomal membrane destabilizer, wherein the endosomal membrane destabilizer is Ca^{++} ion. As set forth in the specification, these methods are based upon the discovery that the presence of endosomal membrane destabilizers, such as Ca^{++} ions, leads to a dramatic increase in the transfection efficiency of nucleic acids (*e.g.*, plasmids) formulated as nucleic acid-lipid particles (*e.g.*, SPLPs or "stabilized plasmid-lipid particles").

II. First Rejection Under 35 U.S.C.§ 103

Claims 1-4, 6-15, 17, 19-22, 27-30, 32-35, 37-46, 48-53, 58- 61 and 63 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious over U.S. Patent No. 5,705,385 ("Bally *et al.*"), taken with either PCT Publication No. WO 98/19710 or U.S. Patent No. 6,177,274 ("Park *et al.*"), and further in view of Lam *et al.* (*J. of Liposome Res.*, 8(4):75-76 (1998)). To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

> As previously explained, as set forth in M.P.E.P. § 2143,
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> [t]o establish a *prima facie* case of obviousness, three basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves of in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure.

In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)

Applicants state that there is simply ***no*** motivation or suggestion provided in the cited references to modify their teaching in the way the Examiner has contemplated. Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion or motivation to do so found either in the reference itself or in the knowledge generally available to one of ordinary skill in the art. *In re Fine*, 837 F.2d 1071, 4 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

Claims 1 and 33 have been amended in accordance with the Examiner's suggestions to expressly recite (1) that the nucleic acid is encapsulated in the lipid; and (2) that the conjugated lipid that inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid, an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula

A——W——Y

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wherein: A is a lipid moiety; W is a hydrophilic polymer; and Y is a polycationic moiety. Amended claim 1 reads as follows:

> Claim 1 (currently amended): A nucleic acid-lipid particle composition for introducing a nucleic acid into a cell, said particle composition comprising:
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> (a) a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles and a nucleic acid, wherein said nucleic acid is encapsulated in the lipid, and wherein said conjugated lipid that inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid, an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula
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> A——W——Y
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> I
>
> wherein:
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> A is a lipid moiety;

W is a hydrophilic polymer; and

Y is a polycationic moiety; and

(b) an endosomal membrane destabilizer, wherein said endosomal membrane destabilizer is Ca^{++} ion.

Bally *et al.* teach an SPLP comprising DODAC/DOPE and PEG-ceramide wherein the nucleic acid is encapsulated in the lipid (*see*, columns 7-9, 11, and 13, Bally *et al.*). In addition, Bally *et al.* disclose that polycationic agents, such as polylysine or salts, can be added to the preformed particle in order to enhance the transfection of the particle to a cell of interest. Bally *et al.* also disclose that PEG or known derivatized PEG-lipids prevent particle aggregation and provide a means for increasing circulation lifetime, thereby increasing the delivery of the lipid-nucleic acid particles to target tissues. Bally *et al.* also disclose that the polynucleotide can be a nucleic acid construct encoding a therapeutic protein, that the average size of the preformed liposome is typically between about 100 nm and several microns, the preferred molecular weight for PEG is about 1000 daltons, and that between 1-15 mole percent of such a derivatized lipid is included in the liposome formulation.

The Examiner correctly acknowledges that Bally *et al.* do ***not*** teach the incorporation of polylysines into the PEG-lipid conjugate, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP compositions of the present invention, e.g., either inside and/or outside of the SPLP (*see*, page 4 of the Office Action).

Park *et al.* teach the use of poly-L-lysine (PLL) as a targeting moiety and a DNA condensate (*see*, column 5, lines 34-47, Park *et al.*). The polylysine used by Park *et al.* is a linear polymer with a MW of 20-30 K. In stark contrast, the present invention utilizes PEG that is a branched chain headgroup made of only a few lysine residues and having a MW of only a few hundred. Moreover, Park *et al.* do ***not*** teach or suggest the use of cationic Ca^{2+} ion as an endosomal disrupting agent in the claimed nucleic acid-lipid particle compositions.

According to the Office Action, one of skill in the art would have been motivated to employ Ca^{++} ions into the presently claimed nucleic acid-lipid particle compositions because "Lam teaches that incorporation of calcium in cationic liposome-plasmid DNA complexes

significantly increases cell transfection in vitro" (*see*, page 5 of the Office Action). As discussed with the Examiner during the telephonic interview, the Lam *et al.* references is directed to cationic liposome-DNA complexes, *i.e.,* lipoplexes, not to the nucleic acid-lipid particle compositions of the present invention, wherein the nucleic acid is encapsulated in the lipid and protected from degradation. As explained above, in accordance with the Examiner's suggestion, the claims (*i.e.*, independent claims 1 and 33) have been amended to more specifically set forth this distinction. It is noted that during the telephonic interview, the Examiner was of the opinion that amending the claims to more sufficiently set forth this distinction, which Applicants have now done, would be sufficient to overcome the obviousness rejection.

In addition to the foregoing, Applicants point out that Haberland *et al.*, which was previously cited by the Examiner, ***teach away*** from enhancing transfection efficiency in cases where cationic liposomes such as Lipofectin and Lipofectamine are used (*see*, page 24, column 2, Haberland *et al.*). Again, according to Haberland *et al.*, "the transfection efficiency of cationic liposomes, such as Lipofectin and Lipofectamine, cannot be reproducibly enhanced by Ca^{2+}" (*see*, page 28, column 1, second paragraph, Haberland *et al.*). As the Examiner is aware, the nucleic acid-lipid particle compositions of the present invention comprise a cationic lipid. As such, taking the teachings of Lam *et al.*, together with the teachings of Haberland *et al.*, one of skill in the art would not have been motivated to use cationic Ca^{2+} ions as an endosomal disrupting agent in the nucleic acid-lipid particle compositions of the present invention since the teachings of Haberland *et al.* lead to a conclusion that is ***opposite*** to the conclusion drawn by Lam *et al.* Again, Haberland *et al.* ***teach away*** from the use of Ca^{2+} to enhance transfection efficiency of cationic liposome-DNA complexes. In any event, neither Lam *et al.* or Haberland *et al.* teach or suggest using cationic Ca^{2+} ions as an endosomal disrupting agent in the nucleic acid-lipid particle compositions of the present invention, wherein the nucleic acid is encapsulated in the lipid and protected from degradation.

In view of the foregoing, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

III. Second Rejection Under 35 U.S.C.§ 103

Claim 1-3, 6-17, 19-30, 32-35, 37-48, 50-61 and 63 remain rejected under 35 U.S.C. § 103(a) as allegedly being obvious in view of U.S. Patent No. 5,705,385 ("Bally *et al.*") taken with PCT Publication No. WO 98/19710 ("Schacht *et al.*"), U.S. Patent No. 6,177,274 ("Park *et al.*") or Lam *et al.* (*J. of Liposome Res.*, 8(4):75-76 (1998)), and in further view of U.S. Patent No. 6,287,591 ("Semple *et al.*"). To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

Again, as noted above, independent claims 1 and 33 have been amended in accordance with the Examiner's suggestions to expressly recite (1) that the nucleic acid is encapsulated in the lipid; and (2) that the conjugated lipid that inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid, an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula

A——W——Y

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wherein: A is a lipid moiety; W is a hydrophilic polymer; and Y is a polycationic moiety.

Bally *et al.* teach an SPLP comprising DODAC/DOPE and PEG-ceramide wherein the DNA is encapsulated in the lipid portion of the SPLP (*see*, columns 7-9, 11, and 13, Bally *et al.*). In addition, Bally *et al.* disclose that polycationic agents, such as polylysine or salts, can be added to the preformed particle in order to enhance the transfection of the particle to a cell of interest. The Examiner acknowledges that the combined cited references of Bally *et al.* taken with either Schacht *et al.*, Park *et al.* or Lam *et al.*, do ***not*** teach that the derivatized lipid-PEG is a diacylglycerolyl based PEG, or that the conjugated lipid comprises a diacyglycerolyl based PEG-polylysine-targeting ligand. Moreover, the Examiner acknowledges that Bally *et al.* do ***not*** teach the incorporation of polylysines into the PEG-lipid conjugate, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the presently claimed SPLP compositions, e.g., either inside and/or outside of the SPLP particle (*see*, pages 6-7 of the Office Action).

Park *et al.* teach the use of poly-L-lysine (PLL) as a targeting moiety and a DNA condensate (*see*, column 5, lines 34-47, Park *et al.*). However, Park *et al.* do ***not*** teach or suggest

the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP particle containing composition of the present invention.

Schacht *et al.* teach synthetic polymer-based carrier vehicles made by self-assembly of the nucleic acid with cationic polymer material so as to condense the nucleic acid and form a polyelectrolyte complex. The complex is then reacted with reactive hydrophilic polymer material which bonds to the complex forming a hydrophilic coating that stabilizes the complex and provides the outer protective steric shield. Membrane-disrupting agents are taught to enable DNA to gain access to the cytoplasm of cells. However, Schacht *et al.* do ***not*** teach or suggest the use of endosomal membrane-disrupting agents in SPLP compositions. Furthermore, Schacht *et al.* do ***not*** teach or suggest the incorporation of polylysines into the PEG-lipid conjugates.

As discussed above in Section II above, the Office Action alleges that Lam *et al.* teach that "incorporation of calcium in cationic liposome-plasmid DNA complexes significantly increases cell transfection in vitro" (*see*, page 5 of the Office Action). However, as discussed with the Examiner during the telephonic interview, the Lam *et al.* references is directed to cationic liposome-DNA complexes, *i.e.,* lipoplexes, not to the nucleic acid-lipid particle compositions of the present invention, wherein the nucleic acid is encapsulated in the lipid and protected from degradation. As explained above, in accordance with the Examiner's suggestion, independent claims 1 and 33 have been amended to more specifically set forth this distinction. It is noted that during the telephonic interview, the Examiner was of the opinion that amending the claims to more sufficiently set forth this distinction between complexes and the nucleic acid-lipid particle compositions of the present invention, which Applicants have now done, would be sufficient to overcome the obviousness rejection. Moreover, as pointed out in Section II, the previously cited Haberland *et al.* reference ***teach away*** from enhancing transfection efficiency in cases where cationic liposomes are used in the transfection process (*see*, page 24, column 2, Haberland *et al.*). Therefore, neither Lam *et al.* nor Haberland *et al.* supplement the deficiencies of Bally *et al.*, Schacht *et al.* and Park *et al.*

Semple *et al.* teach a lipid-therapeutic agent particles containing a charged therapeutic agent encapsulated in lipid portion containing at least two lipid components including a protonatable or deprotonatable lipid such as an amino lipid and a lipid that prevents particle aggregation during lipid-therapeutic agent particle formation, such as a PEG-modified or polyamide oligomer-modified lipid. Semple *et al.* do ***not*** teach or suggest the incorporation of polylysines into the PEG-lipid conjugates, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP compositions. Therefore, Semple *et al.* do not supplement the deficiencies of Bally *et al.*, Schacht *et al.*, Park *et al.* or Lam *et al.* As such, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

VI. Obviousness-Type Double Patenting Rejection

Claims 1-3, 6-17, 19-30, 32-35, 37-48, 50-61 and 63 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 19-63 of U.S. Patent Application No. 09/553,639 ("Cullis *et al.*") taken with Lam *et al.*, as cited above. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

Again, as noted above, independent claims 1 and 33 have been amended in accordance with the Examiner's suggestions to expressly recite (1) that the nucleic acid is encapsulated in the lipid; and (2) that the conjugated lipid that inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid, an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula

A——W——Y

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wherein: A is a lipid moiety; W is a hydrophilic polymer; and Y is a polycationic moiety.

According to the Office Action, the claims of Cullis *et al.* read on lipid-based drug formulations comprising an SPLP comprising a lipid conjugate comprising a diacyglycerolyl based PEG-polylysine-targeting ligand, wherein the polylysine comprises at least for consecutive lysine residues for use in enhancing the delivery of a bioactive agent to cells of

interest. Clearly, Cullis *et al.* do ***not*** teach or suggest the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the claimed SPLP compositions.

The Office Action alleges that Lam *et al.* teach that "incorporation of calcium in cationic liposome-plasmid DNA complexes significantly increases cell transfection in vitro" (*see*, page 5 of the Office Action). However, as discussed with the Examiner during the telephonic interview, the Lam *et al.* references is directed to cationic liposome-DNA complexes, *i.e.,* lipoplexes, not to the nucleic acid-lipid particle compositions of the present invention, wherein the nucleic acid is encapsulated in the lipid and protected from degradation. As explained above, in accordance with the Examiner's suggestion, independent claims 1 and 33 have been amended to more specifically set forth this distinction. It is noted that during the telephonic interview, the Examiner was of the opinion that amending the claims to more sufficiently set forth this distinction between cationic liposome-DNA complexes and the nucleic acid-lipid particle compositions of the present invention, which Applicants have now done, would be sufficient to overcome the obviousness-type double patenting rejection. Moreover, as pointed out in Section II, above, previously cited Haberland *et al.* ***teaches away*** from enhancing transfection efficiency in cases where cationic liposomes are used in the transfection process (*see*, page 24, column 2, Haberland *et al.*). Therefore, Lam *et al.*, either alone or in combination with Haberland *et al.*, does ***not*** supplement the deficiencies of Cullis *et al.*

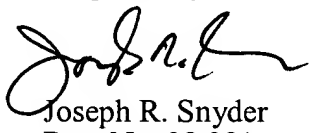
As such, in view of the foregoing, Applicants respectfully request that the obviousness-type double patenting rejection be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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Attachments
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